CLAIMS

(57) [Claim(s)]

[Claim 1] It is the lachrymal matter Lachrymatory Factor under coexistence of PeCSO of the sulfur-containing compound which exists in an onion etc. to enzyme alliinase. It is the lachrymal matter generation enzyme which has the operation to generate. The following physicochemical property:(1) operations; Under coexistence of enzyme alliinase, act on the substrate of the lachrymal matter generation enzyme generated from PeCSO which exists in an onion etc., and generate the thio pro panhard-S-oxide of the lachrymal matter. (2) -- optimal pH; -- pH 5.0-6.0 and (3) molecular-weight; -- the lachrymal matter generation enzyme which has about 18000 (SDS-PAGE electrophoresis method).

[Claim 2] The following physicochemical property:(1) optimum temperature; the lachrymal matter generation enzyme according to claim 1 which has 15-25 degrees C.

[Claim 3] following physicochemical property: -- (1) pH stability; -- the lachrymal matter generation enzyme according to claim 1 which has stability by pH 5.0-9.0 in 15-25 degrees C and the incubation for 10 - 30 minutes.

[Claim 4] following physicochemical property:(1) temperature stability; -- the incubation for pH 6.5 or 5 minutes -- setting -- 60 degrees C or less -- stability and (2) molecular-weight; -- the lachrymal matter generation enzyme according to claim 1 which has about 25000-28000 (FPLC gel filtration technique).

[Claim 5] The manufacture approach of the lachrymal matter generation enzyme according to claim 1 characterized by adding, crushing and extracting water for an onion.

[Claim 6] The lachrymal matter generation enzyme according to claim 1 in which generation of the thio pro panhard-S-oxide in UVlambdahexane (max(e):254(5160) nm) is shown when it measures by the following approaches.

1) Mix this enzyme with alliinase at a suitable rate.

2) Add PeCSO and carry out an enzyme reaction for 1 minute.

3) Add chloroform, **** an enzyme reaction to a stop and coincidence and **** a lachrymal component in a chloroform layer.

4) Measure by HPLC (HPLC: high performance chromatography).

(Conditions)

column: -- silica gel temperature: -- 0-degree-C rate-of-flow: -- 1ml / min mobile phase: -- 2% isopropanol / n-hexane detector: -- UV254nm -- [Claim 7] The measuring method of the activity of the lachrymal matter generation enzyme which is made to add and carry out the enzyme reaction of the PeCSO, and is characterized by measuring generation of thio pro panhard-S-oxide by HPLC (HPLC: high performance chromatography) after mixing a lachrymal matter generation enzyme according to claim 1 with alliinase at a suitable rate.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the new lachrymal matter generation enzyme which generates the lachrymal matter Lachrymatory Factor (henceforth the lachrymal matter LF) generated under coexistence of enzyme alliinase in more detail from PeCSO of the sulfur-containing compound which exists in an onion etc., and its manufacturing method about the enzyme which acts on the precursor generated from PeCSO which exists in an onion etc., and generates the lachrymal matter under coexistence of enzyme alliinase.

[0002]

[Description of the Prior Art] If an onion is cut or mashed, the volatile lachrymal matter LF will be generated. About this lachrymal matter LF in an onion, Virtanen and others The precursor is isolated from an onion and it is (+)-S about this. -(1-propenyl)- L-cysteine sulfoxide (it PeCSO(s)) The matter shown by three is identified among drawing 9 , and it is 1-propenyl sulfenic acid (among drawing 9) about the lachrymal matter LF further. The matter shown by 11 was identified (Spare and C.G.and Virtanen.A.I., Acta Chem.Scand., 17, and 641 (1963)). Then, Brodnitz and others is checking that the lachrymal matter LF is thio pro panhard-S-oxide (matter shown by 12 among drawing 9) by composition (Brodnitz, M.H., and Pascale, J.V., J.Agric.Food.Chem., 19, and 269 (1971)).

[0003] Although the research result of the former many was reported about formation and its decomposition of the lachrymal matter LF in an onion, the generation mechanism of the above-mentioned lachrymal matter LF was considered enzyme alliinase acts on PeCSO of the above-mentioned precursor, and are generated. That is, as a mechanism which the lachrymal matter LF produces, enzyme alliinase acted on PeCSO of a precursor conventionally, and it was thought that it became the nonenzymatic more stable lachrymal matter through a sulfenic acid. However, according to the place which this invention person studied, actually, the above-mentioned component was not produced only in an operation of enzyme alliinase, but it became clear that the intervention of other enzymes is indispensable. Then, while finding out that the new enzyme considered that this invention person isomerizes the above-mentioned sulfenic acid, and generates the lachrymal matter LF as a result of repeating research further wholeheartedly existed, it turned out that the above-mentioned precursor becomes the flavor component of an operation of the enzyme concerned different from the lachrymal matter LF (namely, scent component) or this how therefore.

[0004]

[Problem(s) to be Solved by the Invention] this invention -- the above -- it is completed based on new knowledge and aims at offering the new lachrymal matter generation enzyme which acts on the precursor of the lachrymal matter LF which exists in an onion etc., and generates the lachrymal matter LF concerned, and its manufacture approach.

[0005]

[Means for Solving the Problem] namely, the lachrymal matter generation enzyme which generates the lachrymal matter from PeCSO to which this invention exists in an onion etc. under coexistence of enzyme alliinase -- it comes out.

Moreover, this invention is the lachrymal matter Lachrymatory Factor under coexistence of

PeCSO of the sulfur-containing compound which exists in the following physicochemical property:(1) operation; onions etc. to enzyme alliinase. Have the operation to generate. (2) -- substrate specificity; -- it acts on the precursor generated under coexistence of enzyme alliinase from PeCSO which exists in an onion etc. -- (3) -- optimal pH; -- pH 5.0-6.0 and (4) optimum-temperature; -- 15-25 degrees C Stability [set to 15-25 degrees C and the incubation for 10 - 30 minutes, and] (5) -- pH stability; -- by pH 5.0-9.0 (6) -- temperature stability; -- the incubation for pH 6.5 or 5 minutes -- setting -- 60 degrees C or less -- stability and (7) molecular-weight; -- the aforementioned lachrymal matter generation enzyme which has about 18000 (SDS-PAGE electrophoresis method) and about 25000-28000 (FPLC gel filtration technique) -- it comes out. furthermore, the manufacture approach of the aforementioned lachrymal matter generation enzyme characterized by this invention adding, crushing and extracting water for an onion -- it comes out. moreover, the lachrymal matter generation enzyme in which generation of the thio pro panhard-S-oxide in UVlambdahexane (max(e):254(5160) nm) is shown when this invention is measured by the following approaches -- it comes out.

1) Mix this enzyme with alliinase at a suitable rate.

2) Add PeCSO and carry out an enzyme reaction for 1 minute.

3) Add chloroform, **** an enzyme reaction to a stop and coincidence and **** a lachrymal component in a chloroform layer.

4) Measure by HPLC (HPLC: high performance chromatography).

(Conditions)

column: -- silica gel temperature: -- 0 degree-C rate-of-flow: -- 1ml / min mobile phase: -- 2% isopropanol / n-hexane detector: -- UV -- the measuring method of the activity of the lachrymal matter generation enzyme which is made to add and carry out the enzyme reaction of the PeCSO, and is further characterized by to measure generation of thio pro panhard-S-oxide by HPLC (HPLC: high performance chromatography) 254 nm after this invention mixes a lachrymal matter generation enzyme with alliinase at a suitable rate -- it comes out.

[0006]

[Embodiment of the Invention] Below, this invention is explained further at a detail. Suitably, the enzyme of this invention uses an onion etc. as a raw material, and is extracted and refined, and although manufactured, if it is the above-mentioned enzyme content ingredient, things other than an onion can be used for it like an onion as a raw material. The following approaches are illustrated as a suitable thing as the extract of the enzyme of this invention, and a purification process. That is, for example, an onion is used as a raw material, and it adds water to this with water, and crushes by a mixer etc. Centrifugal [of the obtained debris] is carried out, the supernatant is salted out, and protein is settled. Subsequently, it dissolves in the buffer solutions, such as a phosphoric-acid buffer, centrifugal [of the above-mentioned settlings] is carried out, and the supernatant is extracted as crude enzyme liquid. Here, although various kinds of things can be used as the buffer solution, a potassium phosphate buffer, a citric-acid buffer, an acetic-acid buffer, a tartaric-acid buffer, a succinic-acid buffer,

a maleic-acid buffer, a Tris-HCl buffer, a citric-acid-phosphoric-acid buffer, etc. are illustrated, for example. Next, the crude enzyme liquid obtained by the above-mentioned approach can be made into a purification enzyme preparation by carrying out purification processing, combining suitably means, such as hydroxyapatite, an ammonium-sulfate salting-out, dialysis, the anion exchange, and gel filtration. Although purification of this enzyme from crude enzyme liquid can apply not only the above-mentioned approach but well-known separation and the purification approach, it can obtain crude enzyme protein from crude enzyme liquid with an ammonium-sulfate salting-out method, an organic solvent precipitation method, etc., and can carry out purification processing of this by combining suitably various chromatographies, such as the ion exchange, gel filtration, and an affinity, further, for example.

[0007] This enzyme acts on the precursor generated from PeCSO of the sulfur-containing compound which exists in an onion etc. under coexistence of enzyme alliinase. Since the lachrymal matter LF is generated, work of the enzyme concerned for example, by adjusting by adjustment of pH and temperature conditions, application of an enzyme activity inhibitor, etc. It is possible to control the reaction path of the above-mentioned precursor, and, thereby, the shift to the lachrymal matter LF of a sulfenic acid (namely, scent component) or the shift to another flavor component can be controlled. Therefore, it becomes possible to manufacture onion workpieces, such as onion powder which controls change to a precursor and the lachrymal matter LF from the above PeCSO which exists in an onion by combining enzyme alliinase and this enzyme, and becomes possible [adjusting the flavor of an onion and its workpiece, and stimulative] for example, by which the flavor peculiar to an onion was reinforced, desired flavor, a flavor which has a scent, and a chemical which has a lachrymal operation.

[0008]

[Example] Next, although this invention is concretely explained based on an example, this invention is not limited at all by the example concerned.

It added water to 1l. of distilled water for three extract rough purification onions (790g) of an example 1 (1) enzyme, and crushed by the mixer. With supercentrifuge, centrifugal was carried out for 5 minutes, in addition to [709g of ammonium sulfates] the supernatant (recovery: 1.5l.) (70% saturation), it salted out 8000 rpm, 5 degrees C, and protein was settled. With the supercentrifuge, 8000 rpm, centrifugal was carried out for 5 minutes, and the supernatant was thrown away and dissolved 5 degrees C of settlings in buffer A1 l. (buffer A:pH6.5, 50mM phosphoric-acid K buffer). With supercentrifuge, 12000 rpm, centrifugal was carried out for 15 minutes and 5 degrees C (recovery: 1l.) of supernatants were used as rough purification liquid.

[0009] (2) The hydroxyapatite high-speed rate-of-flow type (Wako) was put in the high RODOKISHI apatite processing 1.5cmx1.5cmx30cm column, and it equilibrated by Buffer A. Subsequently, the liquid (recovery: 900ml) which the above-mentioned rough purification liquid was made to Pass by the 3.5ml of the rates of flow and min, and was made to Pass it was used as Pass liquid.

[0010] (3) In addition to [424.8g of ammonium sulfates] concentration and 900ml of dialysis above-mentioned Pass liquid (70%), it salted out, and protein was settled. With the

supercentrifuge, 12000 rpm, centrifugal was carried out for 5 minutes, and the supernatant was thrown away and dissolved 5 degrees C of settlings in buffer A100 ml. In order to dialyze the above-mentioned solution using the permeable membrane which lets the with a molecular weight of 10000 or less matter pass, buffer A1 l. was used first and it dialyzed under 4 degrees C for 1 hour. The above-mentioned actuation was repeated twice and it dialyzed under 4 degrees C for 17 hours using buffer A1 l. Liquid was made into ejection (recovery: 105ml) from the dialysis tube, and this was made into the liquid after dialysis.

[0011] (4) DE52 (Watt Mann) was put in anion-exchange processing and a concentration 1.0cmx1.0cmx25cm column (78.5ml). It equilibrated by Buffer A and the liquid after dialysis was made to stick to DE52 by the 3.5ml of the rates of flow, and min. Subsequently, it washed by buffer A100 ml and was eluted in the acid K buffer (50mM->500mM:500 ml, 500mM:120 ml) which does not get pH6.5. The result is shown in drawing 1 . Subsequently, the part of the slash of drawing 1 was isolated preparatively (recovery: 120ml), in addition to [56.7g of ammonium sulfates] the liquid isolated preparatively (70%), it salted out, and protein was settled. With the supercentrifuge, 5 degrees C, 20000 rpm, centrifugal was carried out for 5 minutes, and the supernatant was thrown away, dissolved settlings in the buffer A3 milliliter, and used them as the liquid after the anion exchange.

[0012] (5) G-100 (Pharmacia) was put in gel filtration processing and a preservation 0.8cmx0.8cmx70cm column (126ml). It equilibrated by Buffer A and 3ml of liquid after the anion exchange was applied. Buffer A was passed by the 0.33ml of the rates of flow, and min. The result is shown in drawing 2 . Subsequently, the part of the slash of drawing 2 was isolated preparatively (recovery: 25ml), and cryopreservation was carried out at -80 degrees C.

[0013] (6) When the alliinase and PeCSO of the garlic origin in which this enzyme carried out symptom isolation purification are made to react, unless this enzyme exists, don't produce a lachrymal component (thio pro panhard-S-oxide) at all. Existence of this enzyme can be checked by measuring the existence of generation of a lachrymal component by HPLC using this. Moreover, even if it uses the onion origin alliinase which carried out isolation purification by the approach of Mazelis instead of garlic alliinase, a lachrymal component is not produced at all, but in order to aim at the check of this enzyme, it is convenient to use more stable garlic alliinase.

[0014] This enzyme was checked by the following approaches.

1) Mix this enzyme with alliinase at a suitable rate.

2) Add PeCSO and carry out an enzyme reaction for 1 minute.

3) Add chloroform, **** an enzyme reaction to a stop and coincidence and **** a lachrymal component in a chloroform layer.

4) Measure by HPLC (HPLC: high performance chromatography).

(Conditions)

column: -- silica gel temperature: -- 0-degree-C rate-of-flow: -- 1ml / min mobile phase: -- 2% isopropanol / n-hexane detector: -- UV254 -- nm [0015] (7) The enzyme preparation obtained by purification actuation of the property 1 book enzyme of this enzyme of the purity check above checked the single thing by SDS-PAGE electrophoresis. The result of

SDS-PAGE electrophoresis is shown in drawing 3 . The molecular weight of a subunit was about 18000 as a result of SDS-PAGE electrophoresis. Molecular weight was about 25000-28000 as a result of the gel filtration of FPLC. In this invention, measurement of molecular weight based on gel filtration technique is performed using FPLC (Pharmacia manufacture). [0016] 2) The activity measurement enzyme activity of this enzyme was measured by measuring the amount of the generated lachrymal component by HPLC, after optimum dose **** and predetermined made the PeCSO liquid of ****** carry out the time amount reaction of this enzyme and the garlic alliinase simultaneously. In addition, the amount of the generated thio pro panhard-S-oxide was calculated using being UVlambdahexane (max(e):254(5160) nm). moreover, enzyme activity -- per for [reaction] 1 minute -- thio pro panhard-S-oxide -- 1micromol -- the amount of enzymes to produce was defined as 1unit.

[0017] 3) The specific activity in the purification process of the specific activity book enzyme in the purification process of this enzyme was measured. In addition, protein was measured using the Lowry-Folin method. The result is shown in drawing 4 .

[0018] 4) 1ml of these enzymes in which this enzyme carried out temperature stability purification was put into the 1.5ml tube, and it incubated for 1, 3, 5, 10, and 30 minutes on conditions (37 degrees C, 60 degrees C, and 95 degrees C). Subsequently, these were cooled at 0 degree C for 5 minutes, and temperature before a reaction was fixed. It was stable at 37-60 degrees C as a result of activity measurement (drawing 5).

[0019] 5) The 50mM phosphoric acid K buffer of pH stability 3.0, 4.0, 5.0, 6.5, 7.5, and pH 9.0 of this enzyme was produced, and it considered as Buffer B. It incubated at the room temperature in this refined 100micro liter of enzymes for a buffer B900micro liter in addition 10 minutes, and 30 minutes. In pH 5.0-9.0, it was stable at 10 - 30-minute incubation as a result of activity measurement (drawing 6).

[0020] 6) The optimal pH pH conditions of this enzyme were changed, and the strength of the activity when setting pH5.5 to 100 was investigated. The result is shown in drawing 7 . The optimal pH of this enzyme was 5.0-6.0.

[0021] 7) The optimum temperature temperature conditions of this enzyme were changed, and the strength of the activity when setting 20 degrees C to 100 was investigated. The result is shown in drawing 8 . The optimum temperature of this enzyme was 15-25 degrees C. It checked having the physicochemical property which this enzyme described above by the above.

[0022] The various applications which used the operation of this enzyme for below are explained.

It becomes possible to offer an application (1) onion workpiece onion with a gestalt proper as a workpiece in the condition that did not occur the aforementioned enzyme reaction, but enzyme alliinase and this enzyme deactivated, and PeCSO remained. Specifically, the rough fragile article which decorticated thru/or the onion of a hole can be used as the workpiece of the above-mentioned condition by heat-treatment, alcoholic immersion processing, etc. What is necessary is for boiling, a microwave oven, far infrared rays, retorting, etc. just to perform heat-treatment on conditions from which the main temperature of goods of an onion is held 5

minutes or more above 80 degrees C. Alcoholic immersion processing can be performed by being immersed in ethanol etc. about one day. A workpiece can be made into proper gestalten, such as powder which carried out desiccation processing, and a rough fragile article.

[0023] Since the onion powder generally conventionally used for food processing was a thing in the condition that a lifting and PeCSO exhausted the aforementioned enzyme reaction by the production process, it could not generate the lachrymal matter but had the place which cannot present the flavor of onion original, and a scent easily. On the other hand, in the workpiece of the above-mentioned mode, when this is used for food processing etc., the alliinase thru/or this enzyme contained in other raw materials (onion etc.) reacts with PeCSO in a product, and becomes possible [presenting the flavor of onion original, and a scent]. in this case, the inside of the dry product -- alliinase thru/or this enzyme -- each suitable ****** -- it is also possible to blend like, to hydrate at the time of an activity, and to obtain the thing of the above-mentioned quality. As alliinase, the thing of the garlic origin has highly desirable stability. Moreover, by adjusting the amount of each enzyme, it is possible to be able to adjust suitably the quality and the amount of flavor and a scent which are obtained, for example, to advance the reaction of this enzyme, and to consider as a product with more sharp flavor and a scent, and it is possible to stop this and to consider as a mild product.

[0024] Especially the onion powder obtained by the above-mentioned approach has the flavor of onion original, and a scent, and since sweet taste is strong and there is little bitterness, it becomes the outstanding thing which is not in the conventional product as raw materials, such as a stew, Calais, soup of a rahmen, and snack confectionery, and a seasoning, for example. The suitable example of manufacture of the onion powder concerned is shown below.

(Example of manufacture) Three onions (750g) were heated with the microwave oven for 10 minutes, and after adding and crushing 500ml of water, it froze thinly at -80 degrees C. Subsequently, this was dried at 30 degrees C with the freeze dryer for 48 hours, and with a content [of PeCSO] of about 500mg onion powder 50g was obtained.

[0025] (2) An onion flavor can be prepared by making the oil system base distribute the onion flavor PeCSO, alliinase, and this enzyme. In the condition that an oil intervenes, when it does not react, for example, the flavor concerned is used for food etc., hydration of the three above-mentioned components is carried out, and they present desired flavor and a scent. That quality can be suitably adjusted by changing the amount of the three above-mentioned components also in this case.

[0026] (3) The eye lotion can be prepared using the powder prepared like the eye-lotion above (1). Since a lachrymal operation is done so when an enzyme reaction progresses at the time of instillation and the lachrymal matter generates, the eye lotion concerned takes effect for the therapy of a tear deficiency disease (dry eye) etc., for example.

[0027]

[Effect of the Invention] As explained in full detail above, according to this invention, a new lachrymal matter generation enzyme can be offered, and an enzyme can be manufactured by comparatively simple actuation from an onion etc. Since this enzyme has the operation which generates the lachrymal matter LF (namely, scent component) from PeCSO which exists in an

onion etc. under coexistence of enzyme alliinase, it is applicable to an improvement of the flavor of an onion or an onion workpiece etc., for example.

TECHNICAL FIELD

[Field of the Invention] This invention relates to the new lachrymal matter generation enzyme which generates the lachrymal matter Lachrymatory Factor (henceforth the lachrymal matter LF) generated under coexistence of enzyme alliinase in more detail from PeCSO of the sulfur-containing compound which exists in an onion etc., and its manufacturing method about the enzyme which acts on the precursor generated from PeCSO which exists in an onion etc., and generates the lachrymal matter under coexistence of enzyme alliinase.

PRIOR ART

[Description of the Prior Art] If an onion is cut or mashed, the volatile lachrymal matter LF will be generated. About this lachrymal matter LF in an onion, Virtanen and others The precursor is isolated from an onion and it is (+)-S about this. -(1-propenyl)- L-cysteine sulfoxide (it PeCSO(s)) The matter shown by three is identified among drawing 9 , and it is 1-propenyl sulfenic acid (among drawing 9) about the lachrymal matter LF further. The matter shown by 11 was identified (Spare and C.G.and Virtanen.A.I., Acta Chem.Scand., 17, and 641 (1963)). Then, Brodnitz and others is checking that the lachrymal matter LF is thio pro panhard-S-oxide (matter shown by 12 among drawing 9) by composition (Brodnitz, M.H., and Pascale, J.V., J.Agric.Food.Chem., 19, and 269 (1971)).

[0003] Although the research result of the former many was reported about formation and its decomposition of the lachrymal matter LF in an onion, the generation mechanism of the above-mentioned lachrymal matter LF was considered enzyme alliinase acts on PeCSO of the above-mentioned precursor, and are generated. That is, as a mechanism which the lachrymal matter LF produces, enzyme alliinase acted on PeCSO of a precursor conventionally, and it was thought that it became the nonenzymatic more stable lachrymal matter through a sulfenic acid. However, according to the place which this invention person studied, actually, the above-mentioned component was not produced only in an operation of enzyme alliinase, but it became clear that the intervention of other enzymes is indispensable. Then, while finding out that the new enzyme considered that this invention person isomerizes the above-mentioned sulfenic acid, and generates the lachrymal matter LF as a result of repeating research further wholeheartedly existed, it turned out that the above-mentioned precursor becomes the flavor component of an operation of the enzyme concerned different from the lachrymal matter LF (namely, scent component) or this how therefore.

EFFECT OF THE INVENTION

[Effect of the Invention] As explained in full detail above, according to this invention, a new

lachrymal matter generation enzyme can be offered, and an enzyme can be manufactured by comparatively simple actuation from an onion etc. Since this enzyme has the operation which generates the lachrymal matter LF (namely, scent component) from PeCSO which exists in an onion etc. under coexistence of enzyme alliinase, it is applicable to an improvement of the flavor of an onion or an onion workpiece etc., for example.

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] this invention -- the above -- it is completed based on new knowledge and aims at offering the new lachrymal matter generation enzyme which acts on the precursor of the lachrymal matter LF which exists in an onion etc., and generates the lachrymal matter LF concerned, and its manufacture approach.

MEANS

[Means for Solving the Problem] namely, the lachrymal matter generation enzyme which generates the lachrymal matter from PeCSO to which this invention exists in an onion etc. under coexistence of enzyme alliinase -- it comes out.

Moreover, this invention is the lachrymal matter Lachrymatory Factor under coexistence of PeCSO of the sulfur-containing compound which exists in the following physicochemical property:(1) operation; onions etc. to enzyme alliinase. Have the operation to generate. (2) -- substrate specificity; -- it acts on the precursor generated under coexistence of enzyme alliinase from PeCSO which exists in an onion etc. -- (3) -- optimal pH; -- pH 5.0-6.0 and (4) optimum-temperature; -- 15-25 degrees C Stability [set to 15-25 degrees C and the incubation for 10 - 30 minutes, and] (5) -- pH stability; -- by pH 5.0-9.0 (6) -- temperature stability; -- the incubation for pH 6.5 or 5 minutes -- setting -- 60 degrees C or less -- stability and (7) molecular-weight; -- the aforementioned lachrymal matter generation enzyme which has about 18000 (SDS-PAGE electrophoresis method) and about 25000-28000 (FPLC gel filtration technique) -- it comes out. furthermore, the manufacture approach of the aforementioned lachrymal matter generation enzyme characterized by this invention adding, crushing and extracting water for an onion -- it comes out. moreover, the lachrymal matter generation enzyme in which generation of the thio pro panhard-S-oxide in UVlambdahexane (max(e):254(5160) nm) is shown when this invention is measured by the following approaches -- it comes out.

1) Mix this enzyme with alliinase at a suitable rate.

2) Add PeCSO and carry out an enzyme reaction for 1 minute.

3) Add chloroform, **** an enzyme reaction to a stop and coincidence and **** a lachrymal component in a chloroform layer.

4) Measure by HPLC (HPLC: high performance chromatography).

(Conditions)

column: -- silica gel temperature: -- 0 degree-C rate-of-flow: -- 1ml / min mobile phase: -- 2% isopropanol / n-hexane detector: -- UV -- the measuring method of the activity of the lachrymal matter generation enzyme which is made to add and carry out the enzyme reaction of the PeCSO, and is further characterized by to measure generation of thio pro panhard-S-oxide by HPLC (HPLC: high performance chromatography) 254 nm after this invention mixes a lachrymal matter generation enzyme with alliinase at a suitable rate -- it comes out.

[0006]

[Embodiment of the Invention] Below, this invention is explained further at a detail. Suitably, the enzyme of this invention uses an onion etc. as a raw material, and is extracted and refined, and although manufactured, if it is the above-mentioned enzyme content ingredient, things other than an onion can be used for it like an onion as a raw material. The following approaches are illustrated as a suitable thing as the extract of the enzyme of this invention, and a purification process. That is, for example, an onion is used as a raw material, and it adds water to this with water, and crushes by a mixer etc. Centrifugal [of the obtained debris] is carried out, the supernatant is salted out, and protein is settled. Subsequently, it dissolves in the buffer solutions, such as a phosphoric-acid buffer, centrifugal [of the above-mentioned settlings] is carried out, and the supernatant is extracted as crude enzyme liquid. Here, although various kinds of things can be used as the buffer solution, a potassium phosphate buffer, a citric-acid buffer, an acetic-acid buffer, a tartaric-acid buffer, a succinic-acid buffer, a maleic-acid buffer, a Tris-HCl buffer, a citric-acid-phosphoric-acid buffer, etc. are illustrated, for example. Next, the crude enzyme liquid obtained by the above-mentioned approach can be made into a purification enzyme preparation by carrying out purification processing, combining suitably means, such as hydroxyapatite, an ammonium-sulfate salting-out, dialysis, the anion exchange, and gel filtration. Although purification of this enzyme from crude enzyme liquid can apply not only the above-mentioned approach but well-known separation and the purification approach, it can obtain crude enzyme protein from crude enzyme liquid with an ammonium-sulfate salting-out method, an organic solvent precipitation method, etc., and can carry out purification processing of this by combining suitably various chromatographies, such as the ion exchange, gel filtration, and an affinity, further, for example.

[0007] This enzyme acts on the precursor generated from PeCSO of the sulfur-containing compound which exists in an onion etc. under coexistence of enzyme alliinase. Since the lachrymal matter LF is generated, work of the enzyme concerned for example, by adjusting by adjustment of pH and temperature conditions, application of an enzyme activity inhibitor, etc. It is possible to control the reaction path of the above-mentioned precursor, and, thereby, the shift to the lachrymal matter LF of a sulfenic acid (namely, scent component) or the shift to another flavor component can be controlled. Therefore, it becomes possible to manufacture onion workpieces, such as onion powder which controls change to a precursor and the lachrymal matter LF from the above PeCSO which exists in an onion by combining enzyme alliinase and this enzyme, and becomes possible [adjusting the flavor of an onion and its workpiece, and stimulative] for example, by which the flavor peculiar to an onion was

reinforced, desired flavor, a flavor which has a scent, and a chemical which has a lachrymal operation.

EXAMPLE

[Example] Next, although this invention is concretely explained based on an example, this invention is not limited at all by the example concerned.

It added water to 1l. of distilled water for three extract rough purification onions (790g) of an example 1 (1) enzyme, and crushed by the mixer. With supercentrifuge, centrifugal was carried out for 5 minutes, in addition to [709g of ammonium sulfates] the supernatant (recovery: 1.5l.) (70% saturation), it salted out 8000 rpm, 5 degrees C, and protein was settled. With the supercentrifuge, 8000 rpm, centrifugal was carried out for 5 minutes, and the supernatant was thrown away and dissolved 5 degrees C of settlings in buffer A1 l. (buffer A:pH6.5, 50mM phosphoric-acid K buffer). With supercentrifuge, 12000 rpm, centrifugal was carried out for 15 minutes and 5 degrees C (recovery: 1l.) of supernatants were used as rough purification liquid.

[0009] (2) The hydroxyapatite high-speed rate-of-flow type (Wako) was put in the high RODOKISHI apatite processing 1.5cmx1.5cmx30cm column, and it equilibrated by Buffer A. Subsequently, the liquid (recovery: 900ml) which the above-mentioned rough purification liquid was made to Pass by the 3.5ml of the rates of flow and min, and was made to Pass it was used as Pass liquid.

[0010] (3) In addition to [424.8g of ammonium sulfates] concentration and 900ml of dialysis above-mentioned Pass liquid (70%), it salted out, and protein was settled. With the supercentrifuge, 12000 rpm, centrifugal was carried out for 5 minutes, and the supernatant was thrown away and dissolved 5 degrees C of settlings in buffer A100 ml. In order to dialyze the above-mentioned solution using the permeable membrane which lets the with a molecular weight of 10000 or less matter pass, buffer A1 l. was used first and it dialyzed under 4 degrees C for 1 hour. The above-mentioned actuation was repeated twice and it dialyzed under 4 degrees C for 17 hours using buffer A1 l. Liquid was made into ejection (recovery: 105ml) from the dialysis tube, and this was made into the liquid after dialysis.

[0011] (4) DE52 (Watt Mann) was put in anion-exchange processing and a concentration 1.0cmx1.0cmx25cm column (78.5ml). It equilibrated by Buffer A and the liquid after dialysis was made to stick to DE52 by the 3.5ml of the rates of flow, and min. Subsequently, it washed by buffer A100 ml and was eluted in the acid K buffer (50mM->500mM:500 ml, 500mM:120 ml) which does not get pH6.5. The result is shown in drawing 1 . Subsequently, the part of the slash of drawing 1 was isolated preparatively (recovery: 120ml), in addition to [56.7g of ammonium sulfates] the liquid isolated preparatively (70%), it salted out, and protein was settled. With the supercentrifuge, 5 degrees C, 20000 rpm, centrifugal was carried out for 5 minutes, and the supernatant was thrown away, dissolved settlings in the buffer A3 milliliter, and used them as the liquid after the anion exchange.

[0012] (5) G-100 (Pharmacia) was put in gel filtration processing and a preservation 0.8cmx0.8cmx70cm column (126ml). It equilibrated by Buffer A and 3ml of liquid after the anion

exchange was applied. Buffer A was passed by the 0.33ml of the rates of flow, and min. The result is shown in drawing 2 . Subsequently, the part of the slash of drawing 2 was isolated preparatively (recovery: 25ml), and cryopreservation was carried out at -80 degrees C.

[0013] (6) When the alliinase and PeCSO of the garlic origin in which this enzyme carried out symptom isolation purification are made to react, unless this enzyme exists, don't produce a lachrymal component (thio pro panhard-S-oxide) at all. Existence of this enzyme can be checked by measuring the existence of generation of a lachrymal component by HPLC using this. Moreover, even if it uses the onion origin alliinase which carried out isolation purification by the approach of Mazelis instead of garlic alliinase, a lachrymal component is not produced at all, but in order to aim at the check of this enzyme, it is convenient to use more stable garlic alliinase.

[0014] This enzyme was checked by the following approaches.

1) Mix this enzyme with alliinase at a suitable rate.

2) Add PeCSO and carry out an enzyme reaction for 1 minute.

3) Add chloroform, **** an enzyme reaction to a stop and coincidence and **** a lachrymal component in a chloroform layer.

4) Measure by HPLC (HPLC: high performance chromatography).

(Conditions)

column: -- silica gel temperature: -- 0-degree-C rate-of-flow: -- 1ml / min mobile phase: -- 2% isopropanol / n-hexane detector: -- UV254 -- nm [0015] (7) The enzyme preparation obtained by purification actuation of the property 1 book enzyme of this enzyme of the purity check above checked the single thing by SDS-PAGE electrophoresis. The result of SDS-PAGE electrophoresis is shown in drawing 3 . The molecular weight of a subunit was about 18000 as a result of SDS-PAGE electrophoresis. Molecular weight was about 25000-28000 as a result of the gel filtration of FPLC. In this invention, measurement of molecular weight based on gel filtration technique is performed using FPLC (Pharmacia manufacture). [0016] 2) The activity measurement enzyme activity of this enzyme was measured by measuring the amount of the generated lachrymal component by HPLC, after optimum dose **** and predetermined made the PeCSO liquid of ****** carry out the time amount reaction of this enzyme and the garlic alliinase simultaneously. In addition, the amount of the generated thio pro panhard-S-oxide was calculated using being UVlambdahexane (max(e):254(5160) nm). moreover, enzyme activity -- per for [reaction] 1 minute -- thio pro panhard-S-oxide -- 1micromol -- the amount of enzymes to produce was defined as 1unit.

[0017] 3) The specific activity in the purification process of the specific activity book enzyme in the purification process of this enzyme was measured. In addition, protein was measured using the Lowry-Folin method. The result is shown in drawing 4 .

[0018] 4) 1ml of these enzymes in which this enzyme carried out temperature stability purification was put into the 1.5ml tube, and it incubated for 1, 3, 5, 10, and 30 minutes on conditions (37 degrees C, 60 degrees C, and 95 degrees C). Subsequently, these were cooled at 0 degree C for 5 minutes, and temperature before a reaction was fixed. It was stable at 37-60 degrees C as a result of activity measurement (drawing 5).

[0019] 5) The 50mM phosphoric acid K buffer of pH stability 3.0, 4.0, 5.0, 6.5, 7.5, and pH 9.0 of this enzyme was produced, and it considered as Buffer B. It incubated at the room temperature in this refined 100micro liter of enzymes for a buffer B900micro liter in addition 10 minutes, and 30 minutes. In pH 5.0-9.0, it was stable at 10 - 30-minute incubation as a result of activity measurement (drawing 6).

[0020] 6) The optimal pH pH conditions of this enzyme were changed, and the strength of the activity when setting pH5.5 to 100 was investigated. The result is shown in drawing 7 . The optimal pH of this enzyme was 5.0-6.0.

[0021] 7) The optimum temperature temperature conditions of this enzyme were changed, and the strength of the activity when setting 20 degrees C to 100 was investigated. The result is shown in drawing 8 . The optimum temperature of this enzyme was 15-25 degrees C. It checked having the physicochemical property which this enzyme described above by the above.

[0022] The various applications which used the operation of this enzyme for below are explained.

It becomes possible to offer an application (1) onion workpiece onion with a gestalt proper as a workpiece in the condition that did not occur the aforementioned enzyme reaction, but enzyme alliinase and this enzyme deactivated, and PeCSO remained. Specifically, the rough fragile article which decorticated thru/or the onion of a hole can be used as the workpiece of the above-mentioned condition by heat-treatment, alcoholic immersion processing, etc. What is necessary is for boiling, a microwave oven, far infrared rays, retorting, etc. just to perform heat-treatment on conditions from which the main temperature of goods of an onion is held 5 minutes or more above 80 degrees C. Alcoholic immersion processing can be performed by being immersed in ethanol etc. about one day. A workpiece can be made into proper gestalten, such as powder which carried out desiccation processing, and a rough fragile article.

[0023] Since the onion powder generally conventionally used for food processing was a thing in the condition that a lifting and PeCSO exhausted the aforementioned enzyme reaction by the production process, it could not generate the lachrymal matter but had the place which cannot present the flavor of onion original, and a scent easily. On the other hand, in the workpiece of the above-mentioned mode, when this is used for food processing etc., the alliinase thru/or this enzyme contained in other raw materials (onion etc.) reacts with PeCSO in a product, and becomes possible [presenting the flavor of onion original, and a scent]. in this case, the inside of the dry product -- alliinase thru/or this enzyme -- each suitable ****** -- it is also possible to blend like, to hydrate at the time of an activity, and to obtain the thing of the above-mentioned quality. As alliinase, the thing of the garlic origin has highly desirable stability. Moreover, by adjusting the amount of each enzyme, it is possible to be able to adjust suitably the quality and the amount of flavor and a scent which are obtained, for example, to advance the reaction of this enzyme, and to consider as a product with more sharp flavor and a scent, and it is possible to stop this and to consider as a mild product.

[0024] Especially the onion powder obtained by the above-mentioned approach has the flavor of onion original, and a scent, and since sweet taste is strong and there is little bitterness, it

becomes the outstanding thing which is not in the conventional product as raw materials, such as a stew, Calais, soup of a rahmen, and snack confectionery, and a seasoning, for example. The suitable example of manufacture of the onion powder concerned is shown below.

(Example of manufacture) Three onions (750g) were heated with the microwave oven for 10 minutes, and after adding and crushing 500ml of water, it froze thinly at -80 degrees C. Subsequently, this was dried at 30 degrees C with the freeze dryer for 48 hours, and with a content [of PeCSO] of about 500mg onion powder 50g was obtained.

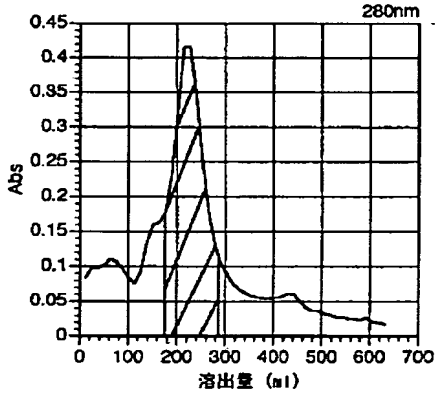
[0025] (2) An onion flavor can be prepared by making the oil system base distribute the onion flavor PeCSO, alliinase, and this enzyme. In the condition that an oil intervenes, when it does not react, for example, the flavor concerned is used for food etc., hydration of the three above-mentioned components is carried out, and they present desired flavor and a scent. That quality can be suitably adjusted by changing the amount of the three above-mentioned components also in this case.

[0026] (3) The eye lotion can be prepared using the powder prepared like the eye-lotion above (1). Since a lachrymal operation is done so when an enzyme reaction progresses at the time of instillation and the lachrymal matter generates, the eye lotion concerned takes effect for the therapy of a tear deficiency disease (dry eye) etc., for example.

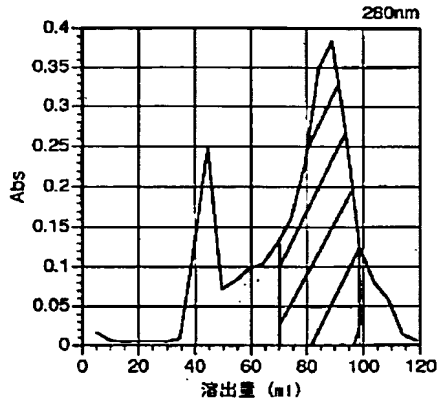
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

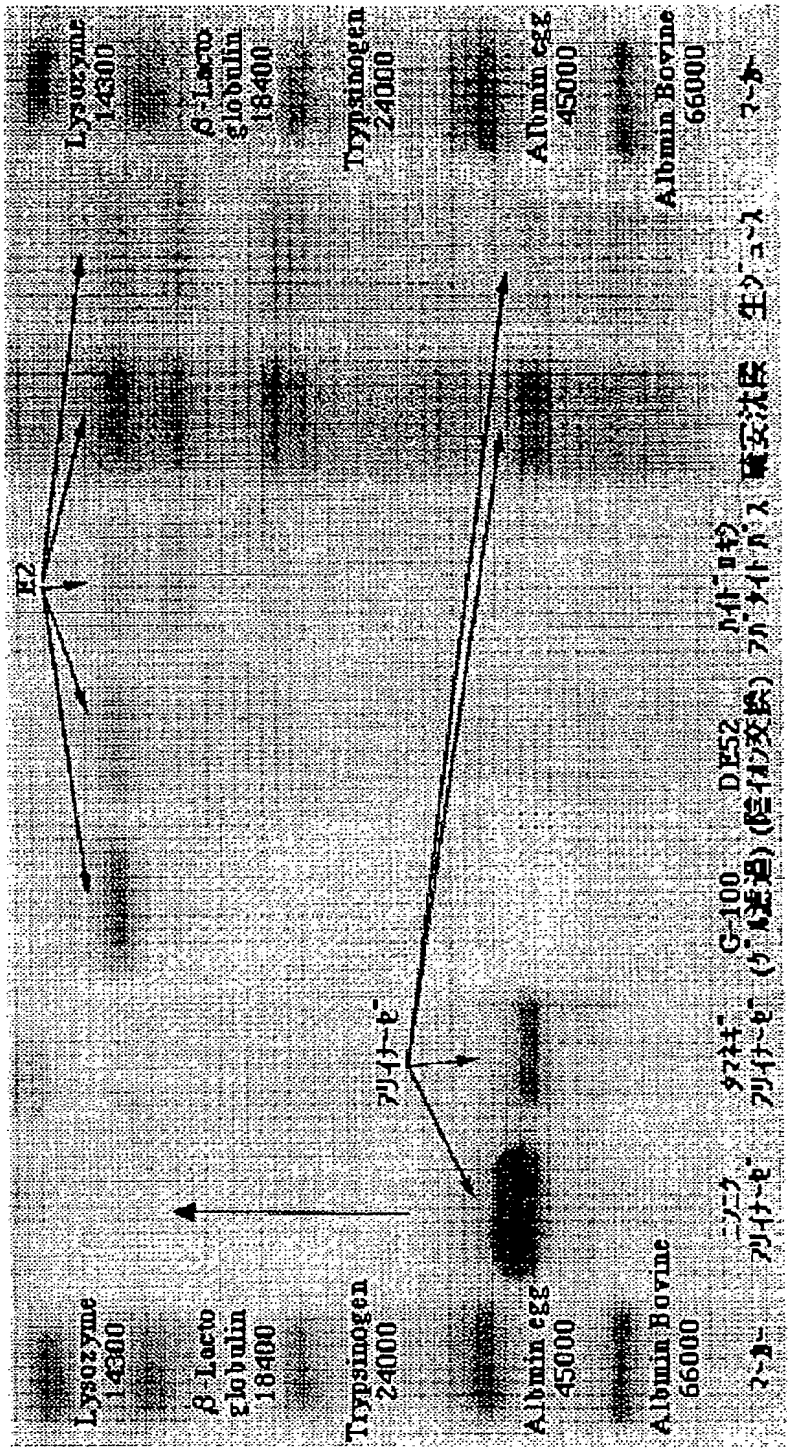
[Drawing 1] The elution pattern of this enzyme (shadow area of drawing) in an anion-exchange chromatography is shown.



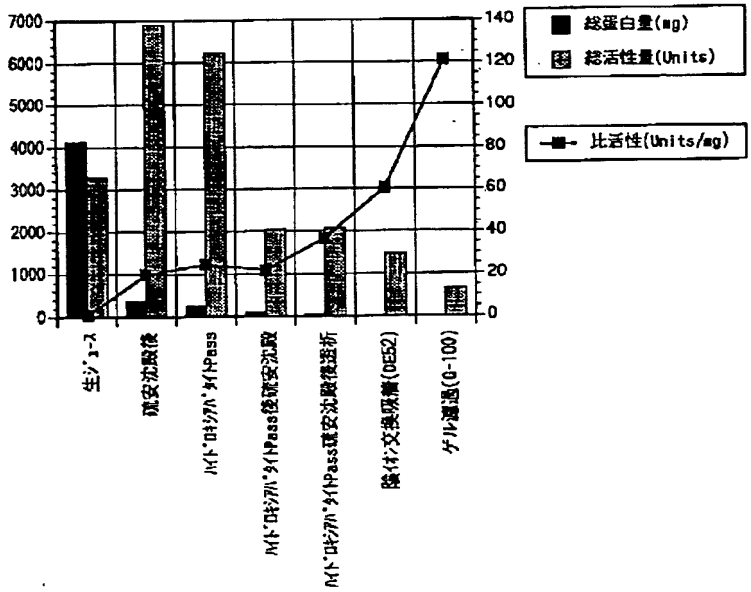
[Drawing 2] The elution pattern of this enzyme (shadow area of drawing) in gel filtration is shown.



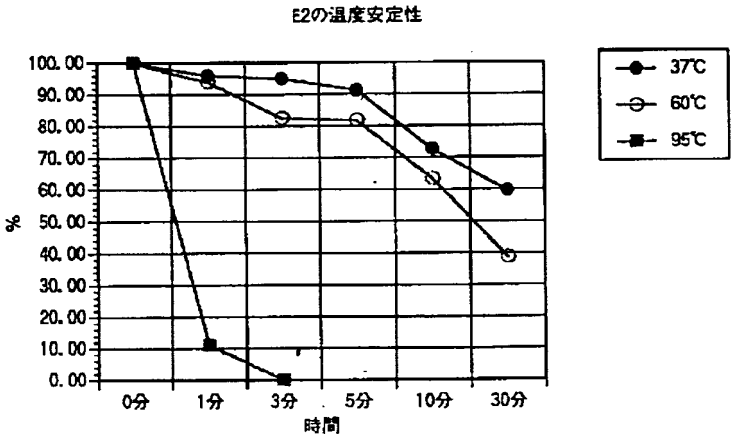
[Drawing 3] The result of SDS-PAGE electrophoresis is shown.



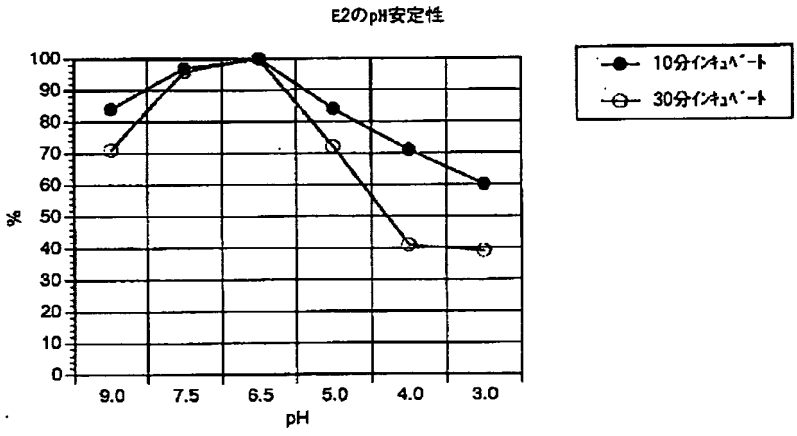
[Drawing 4] The specific activity in the purification process of this enzyme is shown.



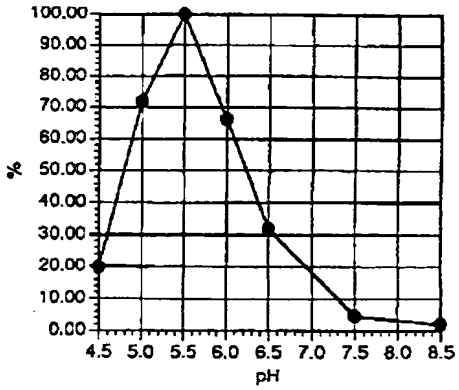
[Drawing 5] The measurement result of the temperature stability of this enzyme is shown.



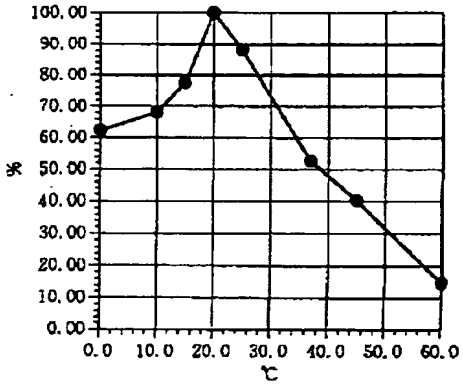
[Drawing 6] The measurement result of the pH stability of this enzyme is shown.



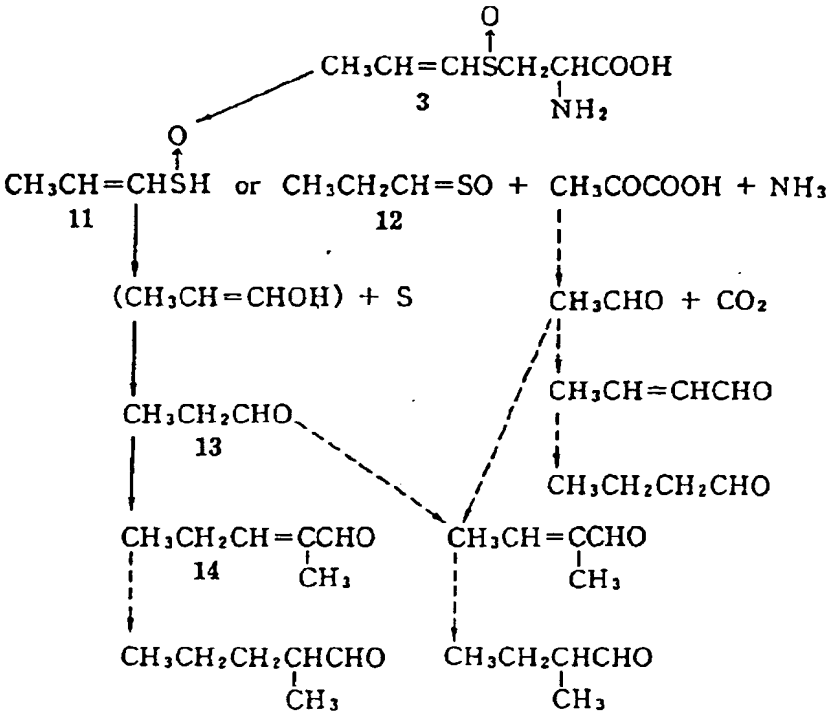
[Drawing 7] The result of having investigated the optimal pH of this enzyme is shown.



[Drawing 8] The result of having investigated the optimum temperature of this enzyme is shown.



[Drawing 9] The explanatory view about formation and decomposition of the lachrymal matter in an onion is shown.



[Description of Notations]

E2: The lachrymal matter generation enzyme of this invention

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最終頁に続く

(54)【発明の名称】　玉葱の催涙性物質生成酵素

(57)【特許請求の範囲】
【請求項１】　玉葱等に存在する含硫化合物のＰｅＣＳＯから酵素アリイナーゼの共存下で催涙性物質Lachrymatory Factor を生成する作用を有する催涙性物質生成酵素であって、以下の理化学的性質：
（１）作用；酵素アリイナーゼの共存下で、玉葱等に存在するＰｅＣＳＯから生成した催涙性物質生成酵素の基質に作用して催涙性物質のチオプロハナール－Ｓ－オキシドを生成する、（２）至適ｐＨ；ｐＨ５．０～６．０、（３）分子量；約１８０００（ＳＤＳ－ＰＡＧＥ電気泳動法）、を有する催涙性物質生成酵素.
【請求項２】　以下の理化学的性質：
（１）至適温度；１５～２５Ｃ、を有する請求項１記載の催涙性物質生成酵素
【請求項３】　以下の理化学的性質：

（１）ｐＨ安定性；１５～２５℃、１０～３０分のインキュベートにおいてｐＨ５．０～９．０で安定、を有する請求項１記載の催涙性物質生成酵素。
【請求項４】　以下の理化学的性質：
（１）温度安定性；ｐＨ６．５、５分のインキュベートにおいて６０℃以下で安定、（２）分子量；約２５００～２８０００（ＦＰＬＣゲル濾過法）、を有する請求項１記載の催涙性物質生成酵素.
【請求項５】　玉葱に水を加えて破砕し、抽出することを特徴とする請求項１記載の催涙性物質生成酵素の製造方法
【請求項６】　以下の方法により測定した場合に、ＵＶλｈｅｘａｎｅ（ｍａｘ（ｅ）：２５４（５１６０）ｎｍ）におけるチオプロハナール－Ｓ－オキシドの生成を示す請求項１記載の催涙性物質生成酵素

1）アリイナーゼと本酵素を適当な割合で混合する。
2）ＰｅＣＳＯを添加し１分間、酵素反応をさせる。
3）クロロフォルムを加えて酵素反応を止め、同時に催涙性成分をクロロフォルム層に転溶する。
4）ＨＰＬＣ（ＨＰＬＣ：高速液体クロマトグラフィー）で測定する。
（条件）
カラム：シリカゲル
温度：０℃
流速：１ミリリットル／ｍｉｎ
移動相：２％イソプロパノール／ｎ－ヘキサン
検出器：ＵＶ２５４ｎｍ

【請求項７】　アリイナーゼと請求項１記載の催涙性物質生成酵素を適当な割合で混合した後、ＰｅＣＳＯを添加して酵素反応させ、チオプロパナール－Ｓ－オキシドの生成をＨＰＬＣ（ＨＰＬＣ：高速液体クロマトグラフィー）で測定することを特徴とする催涙性物質生成酵素の活性の測定方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、酵素アリイナーゼの共存下で、玉葱等に存在するＰｅＣＳＯから生成した前駆物質に作用して催涙性物質を生成する酵素に関するものであり、更に詳しくは、玉葱等に存在する含硫化合物のＰｅＣＳＯから酵素アリイナーゼの共存下で生成される催涙性物質Lachrymatory Factor （以下催涙性物質ＬＦという）を生成する新規催涙性物質生成酵素とその製造法に関するものである。

【０００２】

【従来の技術】玉葱を切断したり、すり潰したりすると揮発性の催涙性物質ＬＦが生成される。玉葱におけるこの催涙性物質ＬＦについて、Virtanenらは、玉葱からその前駆物質の単離を行い、これを（＋）－Ｓ－（１－プロペニル）－Ｌ－システインスルフォキシド（ＰｅＣＳＯ、図９中、３で示される物質）と同定し、更に、催涙性物質ＬＦを１－プロペニルスルフェン酸（図９中、１１で示される物質）と同定した（Spare, C. G. and Virtanen. A. I., Acta Chem. Scand., 17, 641, (1963)）。その後、Brodnitzらは、合成により、催涙性物質ＬＦがチオプロパナール－Ｓ－オキシド（図９中、１２で示される物質）であることを確認している（Brodnitz, M. H., and Pascale, J. V., J. Agric. Food. Chem., 19, 269, (1971)）

【０００３】玉葱における催涙性物質ＬＦの形成及びその分解については、これまで、多くの研究成果が報告されているが、上記催涙性物質ＬＦの生成メカニズムについては、上記前駆物質のＰｅＣＳＯに酵素アリイナーゼが作用して生成されると考えられていた。即ち、催涙性物質ＬＦが生じるメカニスムとして、従来は、前駆物質のＰｅＣＳＯに酵素アリイナーゼが作用し、スルフェン酸を経て非酵素的により安定な催涙性物質になると考えられていた。しかし、本発明者の研究したところによれば、実際に、上記成分は酵素アリイナーゼの作用だけでは生じず、他の酵素の関与が不可欠であることが判明した。そこで、本発明者は更に鋭意研究を積み重ねた結果、上記スルフェン酸を異性化して催涙性物質ＬＦを生成すると考えられる新しい酵素の存在することを見い出すと共に、上記前駆物質は、当該酵素の作用の如何によって、催涙性物質ＬＦ（即ち、香り成分）あるいはこれと別の風味成分になることが分かった。

【０００４】

【発明が解決しようとする課題】本発明は、上記新規な知見に基づいて完成されたものであり、玉葱等に存在する催涙性物質ＬＦの前駆物質に作用して当該催涙性物質ＬＦを生成する新規催涙性物質生成酵素及びその製造方法を提供することを目的とする。

【０００５】

【課題を解決するための手段】即ち、本発明は、酵素アリイナーゼの共存下で玉葱等に存在するＰｅＣＳＯから催涙性物質を生成する催涙性物質生成酵素、である。

また、本発明は、以下の理化学的性質：

（１）作用；玉葱等に存在する含硫化合物のＰｅＣＳＯから酵素アリイナーゼの共存下で催涙性物質Lachrymatory Factor を生成する作用を有する、

（２）基質特異性；酵素アリイナーゼの共存下で、玉葱等に存在するＰｅＣＳＯから生成した前駆物質に作用する、

（３）至適ｐＨ；ｐＨ５．０～６．０、

（４）至適温度；１５～２５℃、

（５）ｐＨ安定性；１５～２５℃、１０～３０分のインキュベートにおいてｐＨ５．０～９．０で安定、

（６）温度安定性；ｐＨ６．５、５分のインキュベートにおいて６０℃以下で安定、

（７）分子量；約１８０００（ＳＤＳ－ＰＡＧＥ電気泳動法）、約２５０００～２８０００（ＦＰＬＣゲル濾過法）、を有する前記の催涙性物質生成酵素、である。更に、本発明は、玉葱に水を加えて破砕し、抽出することを特徴とする前記の催涙性物質生成酵素の製造方法、である。また、本発明は、以下の方法により測定した場合に、ＵＶλｈｅｘａｎｅ（ｍａｘ（ｅ）：２５４（５１６０）ｎｍ）におけるチオプロパナール－Ｓ－オキシドの生成を示す催涙性物質生成酵素、である。

1）アリイナーゼと本酵素を適当な割合で混合する。
2）ＰｅＣＳＯを添加し１分間、酵素反応をさせる。
3）クロロフォルムを加えて酵素反応を止め、同時に催涙性成分をクロロフォルム層に転溶する
4）ＨＰＬＣ（ＨＰＬＣ：高速液体クロマトグラフィー）で測定する
（条件）
カラム：シリカゲル

温度：０℃
流速：１ミリリットル／ｍｉｎ
移動相：２％イソプロパノール／ｎ－ヘキサン
検出器：ＵＶ２５４ｎｍ
更に、本発明は、アリイナーゼと催涙性物質生成酵素を適当な割合で混合した後、ＰｅＣＳＯを添加して酵素反応させ、チオプロパナールＳ－オキシドの生成をＨＰＬＣ（ＨＰＬＣ：高速液体クロマトグラフィー）で測定することを特徴とする催涙性物質生成酵素の活性の測定方法、である。

【０００６】

【発明の実施の形態】以下に、本発明について更に詳細に説明する。本発明の酵素は、好適には玉葱等を原料として、抽出、精製し、製造されるが、原料として、玉葱と同様に、上記酵素含有材料であれば、玉葱以外のものを使用することができる。本発明の酵素の抽出、精製工程として、以下の方法が好適なものとして例示される。即ち、例えば、玉葱を原料とし、これを水で加水し、ミキサー等で破砕する。得られた破砕物を遠心し、その上澄み液を塩析して蛋白質を沈澱させる。次いで、上記沈澱物をリン酸バッファー等の緩衝液に溶解し、遠心し、その上澄み液を粗酵素液として採取する。ここで、緩衝液としては各種のものが使用できるが、例えば、リン酸カリウムバッファー、クエン酸バッファー、酢酸バッファー、酒石酸バッファー、コハク酸バッファー、マレイン酸バッファー、Ｔｒｉｓ－ＨＣｌバッファー、クエン酸－リン酸バッファー等が例示される。次に、上記方法によって得られた粗酵素液を、例えば、ハイドロキシアパタイト、硫安塩析、透析、陰イオン交換、ゲル濾過等の手段を適宜組合わせて、精製処理することにより、精製酵素標品とすることができる。粗酵素液からの本酵素の精製は、上記方法に限らず、公知の分離、精製方法が適用できるが、例えば、粗酵素液から硫安塩析法、有機溶媒沈澱法などにより粗酵素蛋白を得て、更に、これをイオン交換、ゲル濾過、アフィニティー等の各種クロマトグラフィーを適宜組合わせることによって精製処理することができる。

【０００７】本酵素は、酵素アリイナーゼの共存下で玉葱等に存在する含硫化合物のＰｅＣＳＯより生成される前駆物質に作用し、催涙性物質ＬＦを生成することから、当該酵素の働きを、例えば、ｐＨ、温度条件の調整、酵素活性阻害剤の適用等により調整することにより、上記前駆物質の反応経路を制御することが可能であり、これにより、スルフェン酸の催涙性物質ＬＦ（即ち、香り成分）への移行又は別の風味成分への移行をコントロールすることができる。したがって、酵素アリイナーゼと本酵素を組合せることによって、例えば、玉葱に存在する上記ＰｅＣＳＯから前駆物質、催涙性物質ＬＦへの変化を制御して、玉葱及びその加工品の香味と刺激性を調整することが可能となり、例えば、玉葱特有の

香味の増強されたオニオンパウダー、所望の風味、香りを有するフレーバー、催涙作用を有する薬品などの玉葱加工品を製造することが可能となる。

【０００８】

【実施例】次に、実施例に基づいて本発明を具体的に説明するが、本発明は当該実施例によって何ら限定されるものではない。

実施例１

（１）酵素の抽出粗精製

玉葱３個（７９０ｇ）に蒸留水を１リットル加水し、ミキサーで破砕した。高速遠心機で５℃、８０００ｒｐｍ、５分遠心し、その上澄み液（回収：１．５リットル）に硫酸アンモニウム７０９ｇ加え（７０％飽和）塩析し、蛋白質を沈澱させた。高速遠心機で５℃、８０００ｒｐｍ、５分遠心し、上澄み液は捨て、沈澱物をバッファーＡ１リットルに溶解した（バッファーＡ：ｐＨ６．５、５０ｍＭリン酸Ｋバッファー）。高速遠心機で５℃、１２０００ｒｐｍ、１５分遠心し、上澄み液（回収：１リットル）を粗精製液とした。

【０００９】（２）ハイロドキシアパタイト処理

１．５ｃｍ×１．５ｃｍ×３０ｃｍのカラムにハイドロキシアパタイト高速流速タイプ（和光）をつめて、バッファーＡで平衡化した。次いで、上記粗精製液を流速３．５ミリリットル／ｍｉｎでＰａｓｓさせ、Ｐａｓｓさせた液（回収：９００ミリリットル）をＰａｓｓ液とした。

【００１０】（３）濃縮及び透析

上記Ｐａｓｓ液９００ミリリットルに硫酸アンモニウム４２４．８ｇ加え（７０％）塩析し、蛋白質を沈澱させた。高速遠心機で５℃、１２０００ｒｐｍ、５分遠心し、上澄み液は捨て、沈澱物をバッファーＡ１００ミリリットルに溶解した。分子量１００００以下の物質を通す透析膜を用いて上記の溶解液を透析するために、まずバッファーＡ１リットルを用いて４℃下１時間透析した。上記の操作を２回繰り返し、バッファーＡ１リットルを用いて４℃下１７時間透析した。透析チューブから液を取り出し（回収：１０５ミリリットル）、これを透析後液とした。

【００１１】（４）陰イオン交換処理と濃縮

１．０ｃｍ×１．０ｃｍ×２５ｃｍのカラムにＤＥ５２（ワットマン）をつめた（７８．５ミリリットル）。バッファーＡで平衡化し、透析後液を流速３．５ミリリットル／ｍｉｎでＤＥ５２に吸着させた。次いで、バッファーＡ１００ミリリットルで洗浄し、ｐＨ６．５のりん酸Ｋバッファー（５０ｍＭ→５００ｍＭ：５００ミリリットル、５００ｍＭ：１２０ミリリットル）で溶出した。その結果を図１に示す。次いで、図１の斜線の部分を分取して（回収：１２０ミリリットル）、分取した液に硫酸アンモニウム５６．７ｇ加え（７０％）塩析し、蛋白質を沈澱させた。高速遠心機で５℃、２００００ｒ

ｐｍ、５分遠心し、上澄み液は捨て、沈澱物をバッファーＡ３ミリリットルに溶解し、陰イオン交換後液とした。

【００１２】（５）ゲル濾過処理と保存

０．８ｃｍ×０．８ｃｍ×７０ｃｍのカラムにＧ－１００（ファルマシア）をつめた（１２６ミリリットル）。バッファーＡで平衡化し、陰イオン交換後液を３ミリリットルアプライした。流速０．３３ミリリットル／ｍｉｎでバッファーＡを流した。その結果を図２に示す。次いで、図２の斜線の部分を分取して（回収：２５ミリリットル）、－８０℃で凍結保存した。

【００１３】（６）本酵素の確認方法

単離精製したニンニク由来のアリイナーゼとＰｅＣＳＯを反応させた時、本酵素が存在しないと催涙性成分（チオプロパナール－Ｓ－オキシド）は全く生じない。このことを利用してＨＰＬＣで催涙性成分の生成の有無を測定することで、本酵素の存在を確認することができる。また、ニンニクアリイナーゼの代わりに、例えば、Ｍａｚｅｌｉｓの方法によって単離精製した玉葱由来アリイナーゼを用いても催涙性成分は全く生じないが、本酵素の確認を目的とするには、より安定なニンニクアリイナーゼを用いるのが便利である。

【００１４】以下の方法により、本酵素の確認を行った。

１）アリイナーゼと本酵素を適当な割合で混合する。

２）ＰｅＣＳＯを添加し１分間、酵素反応をさせる。

３）クロロフォルムを加えて酵素反応を止め、同時に催涙性成分をクロロフォルム層に転溶する。

４）ＨＰＬＣ（ＨＰＬＣ：高速液体クロマトグラフィー）で測定する。

（条件）

カラム：シリカゲル

温度：０℃

流速：１ミリリットル／ｍｉｎ

移動相：２％イソプロパノール／ｎ－ヘキサン

検出器：ＵＶ２５４ｎｍ

【００１５】（７）本酵素の性質

１）本酵素の純度確認

上記の精製操作によって得られた酵素標品は、ＳＤＳ－ＰＡＧＥ電気泳動で単一であることを確認した。ＳＤＳ－ＰＡＧＥ電気泳動の結果を図３に示す。ＳＤＳ－ＰＡＧＥ電気泳動の結果、サブユニットの分子量は約１８０００であった。ＦＰＬＣのゲル濾過の結果、分子量は約２５０００～２８０００であった。本発明において、ゲル濾過法に基づく分子量の測定は、ＦＰＬＣ（ファルマシア社製）を用いて行う。

【００１６】２）本酵素の活性測定

酵素活性は、適濃度のＰｅＣＳＯ液に本酵素とニンニクアリイナーゼを適時に適量加え、所定の時間反応させた後、生成した催涙性成分の量をＨＰＬＣで測定することによって測定した。尚、生成したチオプロパナール－Ｓ－オキシドの量はＵＶ$\lambda_{max}^{(e)}$：２５４（５１６０）ｎｍ）であることを利用して計算した。また、酵素活性は、反応１分間当りチオプロパナール－Ｓ－オキシドを１μｍｏｌ生じる酵素量を１ｕｎｉｔと定義した。

【００１７】３）本酵素の精製過程における比活性

本酵素の精製過程における比活性を測定した。尚、蛋白質はＬｏｗｒｙ－Ｆｏｌｉｎ法を用いて測定した。その結果を図４に示す。

【００１８】４）本酵素の温度安定性

精製した本酵素１ミリリットルを１．５ミリリットルチューブに入れ３７℃、６０℃、９５℃の条件で１、３、５、１０、３０分インキュベートした。次いで、これらを０℃で５分冷却し反応前の温度を一定にした。活性測定の結果（図５）、３７～６０℃で安定であった。

【００１９】５）本酵素のｐＨ安定性

ｐＨ３．０、４．０、５．０、６．５、７．５、９．０の５０ｍＭりん酸Ｋバッファーを作製し、バッファーＢとした。精製した本酵素１００μリットルにバッファーＢ９００μリットル加えて１０分、３０分室温でインキュベートした。活性測定の結果（図６）、１０～３０分インキュベートでｐＨ５．０～９．０において安定であった。

【００２０】６）本酵素の至適ｐＨ

ｐＨ条件を変えて、ｐＨ５．５を１００としたときの活性の強さを調べた。その結果を図７に示す。本酵素の至適ｐＨは５．０～６．０であった。

【００２１】７）本酵素の至適温度

温度条件を変えて、２０℃を１００としたときの活性の強さを調べた。その結果を図８に示す。本酵素の至適温度は１５～２５℃であった。以上により、本酵素が前記した理化学的性質を有していることを確認した。

【００２２】以下に、本酵素の作用を利用した各種応用例について説明する。

応用例

（１）玉葱加工品

玉葱を、前記の酵素反応を生起せず、酵素アリイナーゼ及び本酵素が失活し、ＰｅＣＳＯが残存した状態の加工品として適宜の形態で提供することが可能となる。具体的には、剥皮した粗破物乃至ホールの玉葱を加熱処理、アルコール浸漬処理等により上記の状態の加工品とすることができる。加熱処理はボイリング、電子レンジ、遠赤外線、レトルト処理等により、玉葱の中心品温が８０℃以上で５分以上保持されるような条件で行なえばよい　アルコール浸漬処理は、エタノール等に１日程浸漬することで行なうことができる　加工品は、乾燥処理したハウダー、粗破物等の適宜の形態とすることができる

【００２３】従来、一般に食品加工に用いられている玉

葱パウダーは、製造工程で前記の酵素反応を起こし、ＰｅＣＳＯが消耗した状態のものであるため、催涙性物質を生成できず、玉葱本来の風味、香りを呈し難い所があった。これに対して、上記態様の加工品では、これを食品加工等に用いた場合に、他の原料（玉葱等）に含まれるアリイナーゼ乃至本酵素が、製品中のＰｅＣＳＯと反応して、玉葱本来の風味、香りを呈することが可能となる。この場合、乾燥した製品の中にアリイナーゼ乃至本酵素を各々適当量含むように配合し、使用時に水和して上記の品質のものを得るようにすることも可能である。アリイナーゼとしてはニンニク由来のものが安定性が高く好ましい。また、各酵素の量を調整することにより、得られる風味、香りの質及び量を適宜調整することができ、例えば、本酵素の反応を進めてより風味、香りがシャープな製品とすることが可能であり、また、これを抑えてマイルドな製品とすることが可能である。

【００２４】特に、上記方法により得られる玉葱パウダーは、玉葱本来の風味、香りを有し、甘味が強く苦味が少ないので、例えば、シチュー、カレー、ラーメンのスープ、スナック菓子等の原料として、また、調味料として従来製品にない優れたものとなる。当該玉葱パウダーの好適な製造例を以下に示す。

（製造例）玉葱３個（７５０ｇ）を電子レンジで１０分加熱し、水５００ミリリットルを加えて破砕した後、－８０℃で薄く凍結した。次いで、これを凍結乾燥機で３０℃で４８時間乾燥して、ＰｅＣＳＯの含量約５００ｍｇの玉葱パウダー５０ｇを得た。

【００２５】（２）玉葱フレーバー

ＰｅＣＳＯ、アリイナーゼ及び本酵素を油系ベースに分散させることにより、玉葱フレーバーを調製することができる。油が介在する状態では上記の３成分は反応せず、例えば、当該フレーバーが食品等に用いられた場合に、水和されて所望の風味、香りを呈する。この場合＊も、上記の３成分の量を変えることによりその品質を適宜調整することができる。

【００２６】（３）目薬

上記（１）と同様にして調製したパウダーを用いて目薬を調製することができる。点眼時に酵素反応が進んで催涙性物質が生成することにより催涙作用が奏されるので、例えば、当該目薬は、涙欠乏症（ドライアイ）等の治療に効果を奏する。

【００２７】

【発明の効果】以上詳述したように、本発明によれば、新規催涙性物質生成酵素を提供することができ、また、酵素を、玉葱等から比較的簡便な操作により製造することができる。本酵素は、酵素アリイナーゼの共存下で玉葱等に存在するＰｅＣＳＯから催涙性物質ＬＦ（即ち、香り成分）を生成する作用を有するので、例えば、玉葱又は玉葱加工品の香味の改善等に利用することができる。

【図面の簡単な説明】

【図１】陰イオン交換クロマトグラフィーにおける本酵素（図の斜線部分）の溶出パターンを示す。

【図２】ゲル濾過における本酵素（図の斜線部分）の溶出パターンを示す。

【図３】ＳＤＳ－ＰＡＧＥ電気泳動の結果を示す。

【図４】本酵素の精製過程における比活性を示す。

【図５】本酵素の温度安定性の測定結果を示す。

【図６】本酵素のｐＨ安定性の測定結果を示す。

【図７】本酵素の至適ｐＨを調べた結果を示す。

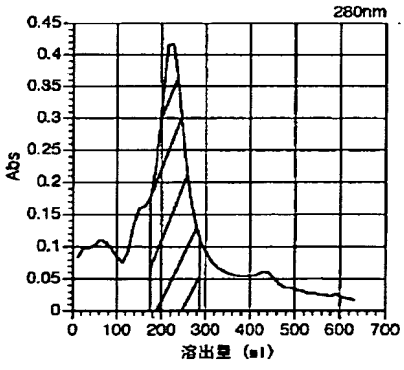
【図８】本酵素の至適温度を調べた結果を示す。

【図９】玉葱における催涙性物質の形成と分解についての説明図を示す。

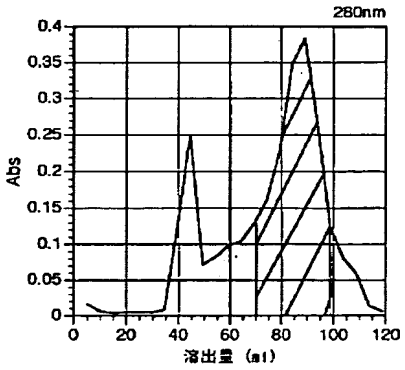
【符号の説明】

Ｅ２：本発明の催涙性物質生成酵素

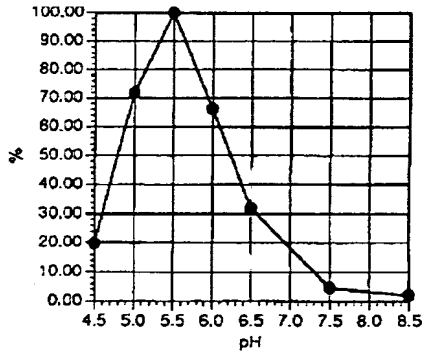
【図１】



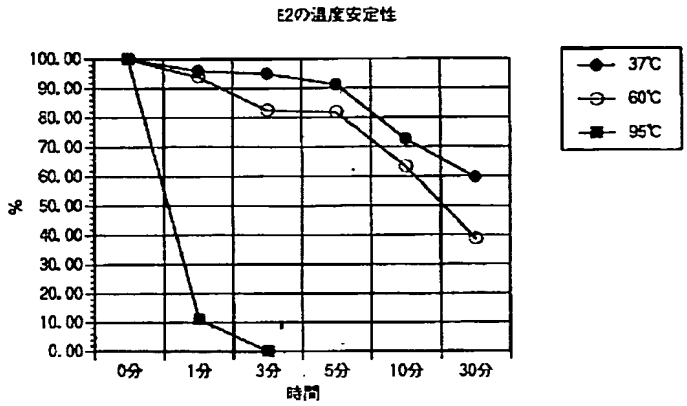
【図２】



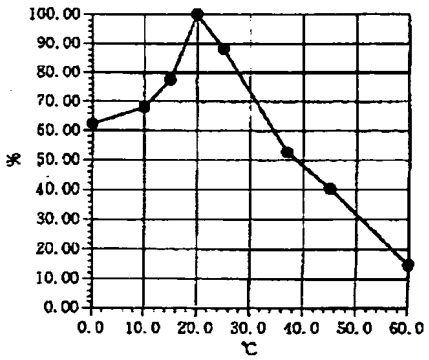
【図７】



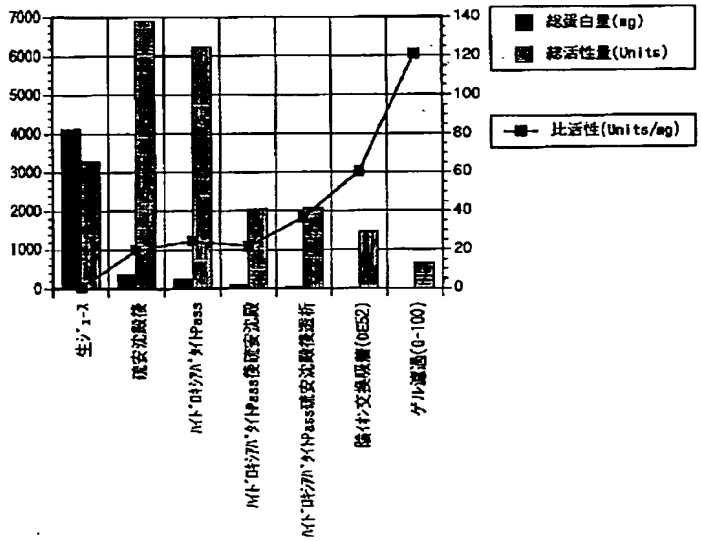
【図５】


E2の温度安定性
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
%
0分
1分
3分
5分
10分
30分
時間
37℃
60℃
95℃

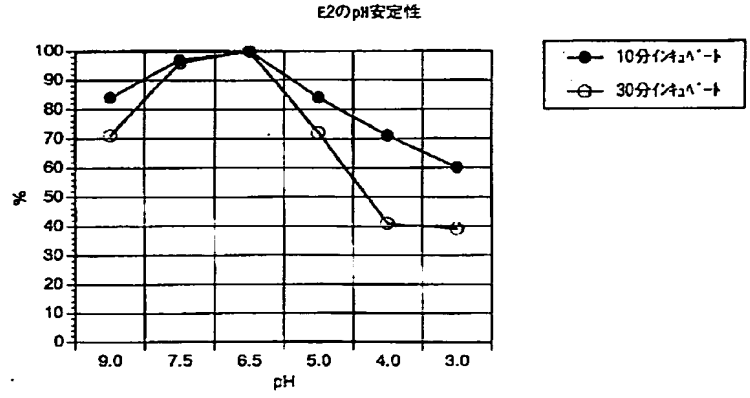
【図８】


100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
%
0.0
10.0
20.0
30.0
40.0
50.0
60.0
℃

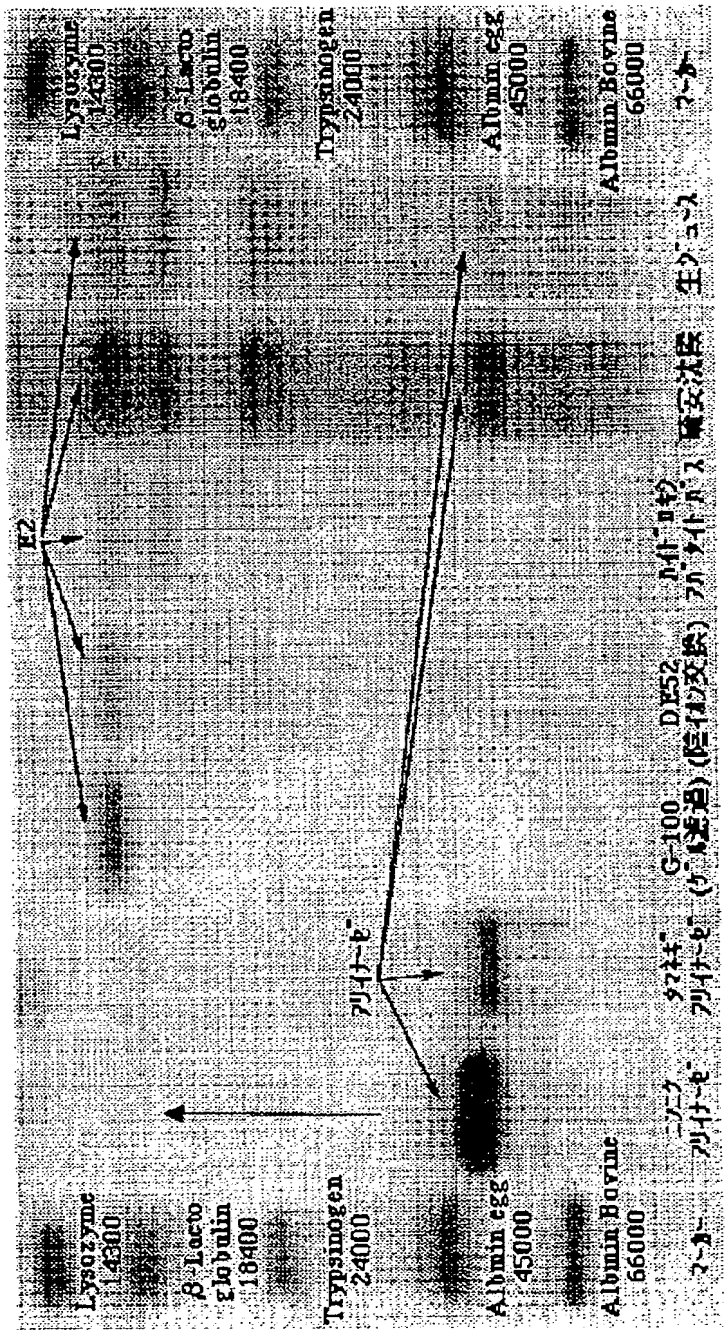
【図４】


7000
6000
5000
4000
3000
2000
1000
0
140
120
100
80
60
40
20
0
総蛋白量(mg)
総活性量(Units)
比活性(Units/mg)
生ジュース
限外濾過後
ハイドロキシアパタイトPass
ハイドロキシアパタイトPass後限外濾過
ハイドロキシアパタイトPass限外濾過後陰イオン交換
陰イオン交換吸着(0ES2)
ゲル濾過(G-100)

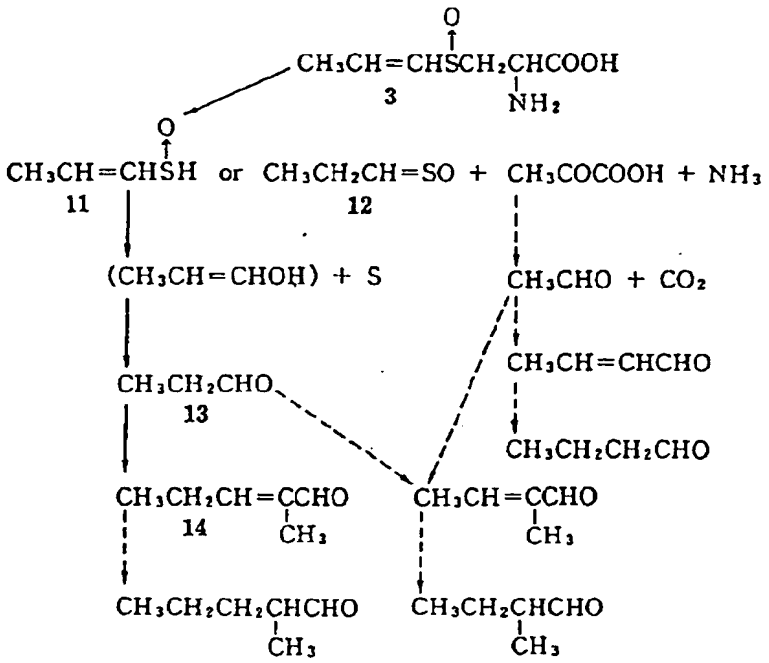
【図６】


E2のpH安定性
100
90
80
70
60
50
40
30
20
10
0
%
9.0
7.5
6.5
5.0
4.0
3.0
pH
10分インキュベート
30分インキュベート

【図３】


Lysozyme
14300
β-Lacto
globulin
18400
Trypsinogen
24000
Albmin egg
45000
Albmin Bovine
66000
E2
G-100
DE52
Lysozyme
14300
β-Lacto
globulin
18400
Trypsinogen
24000
Albmin egg
45000
Albmin Bovine
66000

【図９】



フロントページの続き

審査官　冨永　みどり

(58)調査した分野(Int.Cl.⁷, ＤＢ名)
C12N 9/00 - 9/99
ＢＩＯＳＩＳ／ＷＰＩ（ＤＩＡＬＯＧ）